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(54) Title: THERAPEUTIC METHODS COMPRISING USE OF A NEUREGULIN

(57) Abstract

The invention provides methods for treatment and/or prophylaxis of certain neurological-related disorders, particularly treatment or prophylaxis of the effects of stroke, brain or spinal cord injury or ischemia, heart attack, optic nerve and retinal injury and ischemia and other acute-type conditions disclosed herein as well as chronic-type conditions, specifically epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Down's Syndrome, Korsakoff's disease, cerebral palsy and/or age-dependent dementia. The methods of the invention comprise administration of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a neuregulin fragment or derivative, to a patient suffering from or susceptible to such conditions.

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THERAPEUTIC METHODS COMPRISING USE OF A NEUREGULIN

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to methods for treatment of certain neurological-related injuries and disorders comprising use of a neuregulin, or a fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or neuregulin fragment or derivative.

2. Background

Nerve cell death (degeneration) can cause potentially devastating and irreversible effects for an individual and may occur e.g. as a result of stroke, heart attack or other brain or spinal chord ischemia or trauma. Additionally, neurodegenerative disorders involve nerve cell death (degeneration) such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Down's Syndrome and Korsakoff's disease.

Therapies have been investigated to treat nerve cell degeneration and related disorders, e.g., by limiting the extent of nerve cell death that may otherwise occur to an individual as well as promoting repair, remodeling and reprogramming after stroke or other neuronal injury. See, e.g., F. Seil, *Curr Opin Neuro*, 10:49-51 (1997); N. L. Reddy et al., *J Med Chem*, 37:260-267 (1994); and WO 95/20950.

Certain growth factors have been reported to exhibit neuroprotective properties. In particular, nerve growth factor (NGF) has been evaluated in certain neuroprotective models. See, for example, G. Sinson et al., *J Neurosurg*, 86(3):511-518 (1997); and G. Sinson et al., *J Neurochem*, 65(5):2209-2216 (1995). Osteogenic protein-1 (OP-1) has been evaluated in a rat model of cerebral hypoxia/ischemia for neuroprotective activity. G. Perides, *Neurosci Lett*, 1871):21-24 (1995). Glial cell line-derived neurotrophic factor (GDNF) was reported to exhibit trophic activity on certain populations of central neurons. Y. Wang et al., *J Neurosci*, 17(11):4341-4348 (1997). Small molecules also have been investigated as neuroprotective agents, such

as MK-801. See B. Meldrum , *Cereb Brain Metab Rev*, 2:27-57 (1990); D. Choi, *Cereb Brain Metab Rev*, 2:27-57 (1990).

However, no effective pharmacotherapies are in regular clinical use for ischemia-induced brain injury or other such injuries and disorders. See, for example, Y. Wang et al., *supra*; G. Sinson et al., *J Neurochem*, *J Neurochem*, 65(5):2209 (1995).

It thus would be highly desirable to have new neuroprotective agents, particularly agents to limit the extent or otherwise treat nerve cell death (degeneration) that occur with stroke, heart attack or brain or spinal cord trauma, or to treat Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Down's Syndrome and Korsakoff's disease. It also would be desirable to have agents that promote repair, remodeling or reprogramming after stroke or other neuronal injury.

SUMMARY OF THE INVENTION

The present invention provides methods for treatment and/or prophylaxis of certain neurological-related disorders, particularly treatment or prophylaxis of the effects of stroke, brain or spinal cord injury or ischemia, heart attack, optic nerve and retinal injury and ischemia and other acute-type conditions disclosed herein as well as chronic-type conditions, specifically epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Down's Syndrome, Korsakoff's disease, cerebral palsy and/or age-dependent dementia. Methods of the invention also include therapies for promoting repair, remodeling or reprogramming after stroke or other neuronal injury.

The methods of the invention comprise administration of an effective amount of neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a neuregulin fragment or derivative (i.e. gene therapy), to a patient suffering from or susceptible to such conditions.

Neuregulins are members of the epidermal growth factor (EGF) superfamily and include glial growth factor (GGF), acetylcholine receptor-inducing activity (ARIA), neu differentiation factor (NDF) and heregulins (HRF). See D. E. Wen et al., *Cell*, 69:559-572 (1992); W.E. Holmes et al., *Science*, 256:1205-1210 (1992); M.A. Marchionni et al., *Nature*, 362:312-318 (1993); and D.L. Falls, *Cell*, 72:801-815

(1993). A variety of neuregulins and fragments and derivatives thereof can be employed in the methods of the invention. For example, suitable agents have been disclosed in U.S. Patent 5,530,109 and PCT/US93/07491. Neuregulins also have been reported in U.S. Patent 5,367,060. Preferred neuregulins include regions shown in FIGS. 1-2 (SEQ ID NOS. 2 and 4), also known as the E sequence. Preferred neuregulins or fragments or derivatives also include those that contain the C, C/D or C/D' sequences as shown in Figures 7, 8 and 9 respectively of the drawings, or those neuregulins or fragments or derivatives that have substantial homology to the peptide sequences shown in Figures 7, 8 or 9, e.g. at least about 70 percent homology, or at least about 80 percent homology, or more preferably at least about 90 or 95 percent homology to the peptide sequences shown in Figures 7, 8 or 9. Preferred nucleic acids and fragments and derivatives for use in the methods of the invention include those nucleic acids that include one or more nucleic acids sequences shown in Figures 7, 8 and 9 of the drawings, or those nucleic acids that that have substantial homology to the nucleic acid sequences shown in Figures 7, 8 or 9, e.g. at least about 70, 80, 90 or 95 percent homology to the nucleic acid sequences shown in Figures 7, 8 or 9. A particularly preferred neuregulin is encoded by DNA obtainable from the clone pGGF2HBS11 (ATCC Deposit No. 75347). Also preferred are neuregulins encoded by DNA obtainable from GGF2BPP5, GGF2BPP2 and GGF2BPP4.

Typical patients that may be treated in accordance with the methods of the invention are persons suffering from brain or spinal cord trauma or ischemia, stroke, heart attack, hypoxia, hypoglycemia, post-surgical neurological deficits, decreased blood flow or nutrient supply to retinal tissue or optic nerve, retinal trauma or ischemia or optic nerve injury. Patients suffering from chronic-type conditions also may be treated in accordance with the invention, specifically subjects suffering from or susceptible to epilepsy, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Alzheimer's disease, Down's Syndrome, Korsakoff's disease, cerebral palsy and/or age-dependent dementia.

Also, as discussed above, a neuregulin or fragment or derivative thereof or nucleic acid encoding same, may be administered to promote repair, remodeling or reprogramming to a subject that has suffered stroke or other neuronal injury such as traumatic brain or spinal cord injury. In such cases, the therapeutic agent may be

suitably administered to the subject over an extended period following the injury, e.g. at least about 1, 2, 3, 4, 6, 8, 12 or 16 weeks following the injury.

Other aspects of the invention are disclosed *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a nucleotide sequence (SEQ ID NO:1) encoding a preferred neuregulin region (E segment of human GGF) and the amino acid sequence (SEQ ID NO:2) of that preferred region.

FIG. 2 shows a nucleotide sequence (SEQ ID NO:3) encoding a preferred neuregulin region (E segment of bovine GGF) and the amino acid sequence (SEQ ID NO:4) of that preferred region.

FIG. 3 shows nucleotide sequences (SEQ ID NOS:6-7) encoding further neuregulin regions (B segment of human and bovine GGF) and amino acid sequences (SEQ ID NOS:5 and 8) of those regions. Line 1 is the predicted amino acid sequence of bovine B segment, line 2 is a nucleotide sequence of bovine B segment, line 3 is a nucleotide sequence of human B segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human B segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 4 shows nucleotide sequences (SEQ ID NOS:10-11) encoding further neuregulin regions (A segment of human and bovine GGF) and amino acid sequences (SEQ ID NOS:9 and 12) of those regions. Line 1 is the predicted amino acid sequence of bovine A segment, line 2 is a nucleotide sequence of bovine A segment, line 3 is a nucleotide sequence of human A segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human A segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 5 shows a nucleotide sequence (SEQ ID NO:13) encoding a further neuregulin region (A' segment of bovine GGF) and the predicted amino acid sequence (SEQ ID NO:14) of that region.

FIG. 6 shows nucleotide sequences (SEQ ID NOS:16-17) encoding further neuregulin regions (G segment of bovine and human GGF) and amino acid sequences (SEQ ID NOS:15 and 18) of that region. Line 1 is the predicted amino acid sequence of bovine G segment, line 2 is a nucleotide sequence of bovine G segment, line 3 is a

nucleotide sequence of human G segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human G segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 7 shows nucleotide sequences (SEQ ID NOS:20-21) encoding further neuregulin regions (C segment of bovine and human GGF) and amino acid sequences (SEQ ID NOS:19 and 22) of those regions. Line 1 is the predicted amino acid sequence of bovine C segment, line 2 is a nucleotide sequence of bovine C segment, line 3 is a nucleotide sequence of human C segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human C segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 8 shows nucleotide sequences (SEQ ID NOS:24-25) encoding further neuregulin regions (C/D segment of human and bovine GGF) and amino acid sequences (SEQ ID NOS:23 and 26) of those regions. Line 1 is the predicted amino acid sequence of bovine C/D segment, line 2 is a nucleotide sequence of bovine C/D segment, line 3 is a nucleotide sequence of human C/D segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human C/D segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 9 shows nucleotide sequences (SEQ ID NOS:28-29) encoding a further neuregulin region (C/D' segment of the human and bovine GGF) and the amino acid sequence (SEQ ID NO:27) of that region. Line 1 is the predicted amino acid sequence of the C/D' segment, line 2 is a nucleotide sequence of bovine C/D' segment and line 3 is a nucleotide sequence of human C/D' segment (nucleotide base matches are indicated with a vertical line).

FIG. 10 shows nucleotide sequences (SEQ ID NOS:31-32) encoding a further neuregulin region (D segment of the human and bovine GGF) and the amino acid sequence (SEQ ID NO:30) of that region. Line 1 is the predicted amino acid sequence of the D segment, line 2 is a nucleotide sequence of bovine D segment and line 3 is a nucleotide sequence of human D segment (nucleotide base matches are indicated with a vertical line).

FIG. 11 shows nucleotide sequence (SEQ ID NO:34) encoding a further neuregulin region (D' segment of bovine GGF) and the amino acid sequence (SEQ ID NO:33) of that region.

FIGS. 12A-12B show nucleotide sequences (SEQ ID NOS:36-37) encoding further neuregulin regions (H segment of human and bovine GGF) and amino acid sequences (SEQ ID NO:35 and 38) of that region. Line 1 is the predicted amino acid sequence of bovine H segment, line 2 is a nucleotide sequence of bovine H segment, line 3 is a nucleotide sequence of human H segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human H segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 13 shows a nucleotide sequence (SEQ ID NO:40) encoding a further neuregulin region (K segment of bovine GGF) and the amino acid sequence (SEQ ID NO:39) of that region.

FIGS. 14A-14C show nucleotide sequences (SEQ ID NOS:42-43) encoding a further neuregulin region (L segment of bovine and human GGF) and amino acid sequences (SEQ ID NO:41 and 44) of that region. Line 1 is the predicted amino acid sequence of bovine L segment, line 2 is a nucleotide sequence of bovine L segment, line 3 is a nucleotide sequence of human L segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human L segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 15 shows nucleotide sequences (SEQ ID NOS:46-47) encoding further neuregulin regions (F segment of bovine and human GGF) and amino acid sequences (SEQ ID NOS:45 and 48) of that region. Line 1 is the predicted amino acid sequence of bovine F segment, line 2 is a nucleotide sequence of bovine F segment, line 3 is a nucleotide sequence of human F segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human F segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIGS. 16A-16C show the nucleotide sequence (SEQ ID NO:49) and deduced amino acid sequence (SEQ ID NO:50) of GGF2BPP4.

FIGS. 17A-17B show the nucleotide sequence (SEQ ID NO:51) and deduced amino acid sequence (SEQ ID NO:52) of GGF2BPP2.

FIGS. 18A-18B show the nucleotide sequence (SEQ ID NO:53) and deduced amino acid sequence (SEQ ID NO:54) of GGF2BPP5.

DETAILED DESCRIPTION OF THE INVENTION

As discussed above, preferred neuregulins for use in the therapeutic methods of the present invention include those disclosed in U.S. Patent 5,530,109 and PCT/US93/07491, incorporated herein by reference. Particularly preferred neuregulins comprise an amino acid sequence of the following formula:

WYBAZCX

wherein WYBAZCX is composed of amino acid sequences that include one or more sequences shown in FIGS. 1 through 15 (which includes SEQ ID NOS:2, 4, 5, 8, 9, 12, 14, 15, 18, 19, 22, 23, 26, 27, 30, 33, 35, 38, 39, 41, 44, 45 and 48), wherein W comprises the polypeptide segment F, or is absent; wherein Y comprises the polypeptide segment E, or is absent; wherein Z comprises the polypeptide segment G or is absent; and wherein X comprise a polypeptide segment selected from the group consisting of C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/d D'H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL and C/D C/D' D' HKL, and preferably that either

a) at least one of F, Y, B, A, Z, C or X is of bovine origin; or

b) Y comprises the polypeptide segment E; or

c) X comprises the polypeptide segments C/D HKL, C/D D, C/D' HKL, C/D C/D' HKL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HKL, C/D C/D' D'H, C/D C/D' D HL, C/D C/D' D' HKL, C/D'H, C/D C/D' H or C/D C/D' HL.

Particularly preferred neuregulins also include those polypeptides that include the segments FB polypeptides that include the segments FBA' (i.e. the groups F, B and A' as defined herein including in the drawings); polypeptides that include the segments EBA (i.e. the groups E, B and A as defined herein including in the drawings); polypeptides that include the segments EBA' (i.e. the groups E, B and A' as defined herein including in the drawings); A (i.e. the group A as defined herein

including in the drawings); polypeptides that include the segments FEBA (i.e. the groups F, E, B and A as defined herein including in the drawings); polypeptides that include the segments FBA' (i.e. the groups F, B and A' as defined herein including in the drawings); and polypeptides that include the segments FEBA' (i.e. the groups F, E, B and A' as defined herein including in the drawings).

Also preferred are nucleic acids that code for the above preferred polypeptides.

A "fragment" or "derivative" of a neuregulin refers to herein 1) a peptide in which one or more amino acid residues are with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) a peptide in which one or more of the amino acid residues includes a substituent group, or (iii) a peptide in which the mature protein is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol). Thus, a fragment or derivative for use in accordance with the methods of the invention includes a proprotein, which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide fragments and derivatives of the invention are of a sufficient length to uniquely identify a region of a neuregulin. Neuregulin fragments thus preferably comprise at least 8 amino acids, usually at least about 12 amino acids, more usually at least about 15 amino acids, still more typically at least about 30 amino acids, even more typically at least about 50 or 70 amino acids. Preferred fragments or derivatives for use in the methods of the invention include those that have at least about 70 percent homology (sequence identity) to any of the preferred sequences mentioned above, more preferably about 80 percent or more homology to any of the preferred sequences mentioned above, still more preferably about 85 to 90 percent or more homology to any of the preferred sequences mentioned above. Sequence identity or homology with respect to a neuregulin as referred to herein is the percentage of amino acid sequences of a neuregulin protein or fragment or derivative thereof that are identical with a specified sequence, after introducing any gaps necessary to achieve the maximum percent homology.

The neuregulin fragments and derivatives for use in the methods of the invention preferably exhibit good activity in standard neuroprotective assays such as

the *in vivo* cerebral ischemia assay of Example 1, which follows. That assay includes the following steps: a) continuous intraventricular infusion of the protein fragment or derivative or vehicle alone to test rats for three days prior to inducing focal ischemic infarcts in right lateral cerebral cortex; and b) twenty-four hours after inducing ischemic infarcts, infarct volume in each test animal is determined by image analysis. Preferably, a protein fragment or derivative of the invention provides at least about a 10% reduction in infarct volume relative to vehicle-treated animals, more preferably about a 20% reduction in infarct volume, still more preferably about a 25% reduction in infarct volume relative to vehicle-treated animals in such an assay. References herein to *in vivo* cerebral ischemia assay are intended to refer to an assay of the above steps a) and b), which are more fully described in Example 1 which follows.

As discussed above, neuregulin nucleic acid fragments and derivatives are also provided for use in the methods of the invention. Those fragments and derivatives typically are of a length sufficient to bind to a sequence of any of the nucleic acid sequences shown in Figures 1-15 of the drawings, including SEQ ID NOS:1, 3, 6, 7, 10, 11, 13, 16, 17, 20, 21, 24, 25, 28, 29, 31, 32, 34, 36, 37, 40, 42 and 43 under the following moderately stringent conditions (referred to herein as "normal stringency" conditions): use of a hybridization buffer comprising 20% formamide in 0.8M saline/0.08M sodium citrate (SSC) buffer at a temperature of 37°C and remaining bound when subject to washing once with that SSC buffer at 37°C.

Preferred neuregulin nucleic acid fragments and derivatives of the invention will bind to a sequence of any of the nucleic acid sequences shown in Figures 1-15 of the drawings, including SEQ ID NOS:1, 3, 6, 7, 10, 11, 13, 16, 17, 20, 21, 24, 25, 28, 29, 31, 32, 34, 36, 37, 40, 42 and 43 under the following highly stringent conditions (referred to herein as "high stringency" conditions): use of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M sodium citrate (SSC) buffer at a temperature of 42°C and remaining bound when subject to washing twice with that SSC buffer at 42°C.

The neuregulin nucleic acid fragments and derivatives preferably should comprise at least 20 base pairs, more preferably at least about 50 base pairs, and still more preferably a nucleic acid fragment or derivative of the invention comprises at least about 100, 200, 300 or 400 base pairs. In some preferred embodiments, the

nucleic acid fragment or derivative is bound to some moiety which permits ready identification such as a radionucleotide, fluorescent or other chemical identifier.

Isolated neuregulin and peptide fragments or derivatives of the invention are preferably produced by recombinant methods, although suitable neuregulins also can be isolated from various sources. See the procedures disclosed U.S. Patent 5,530,109; U.S. Patent 5,367,060; and PCT/US93/07491, incorporated herein by reference. A wide variety of molecular and biochemical methods are available for generating and expressing neuregulin; see e.g. the procedures disclosed in *Molecular Cloning, A Laboratory Manual* (2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor), *Current Protocols in Molecular Biology* (Eds. Aufubel, Brent, Kingston, More, Feidman, Smith and Stuhl, Greene Publ. Assoc., Wiley-Interscience, NY, N.Y. 1992) or other procedures that are otherwise known in the art. For example, neuregulin or fragments or derivatives thereof may be obtained by chemical synthesis, or more preferably by expression in bacteria such as *E coli* and eukaryotes such as yeast, baculovirus, or mammalian cell-based expression systems, etc., depending on the size, nature and quantity of neuregulin or fragment or derivative thereof. More particularly, a recombinant DNA molecule comprising a vector and a DNA segment encoding neuregulin, or a fragment or derivative thereof, can be constructed. Suitable vectors include e.g. baculovirus-derived vectors for expression in insect cells (see Pennock et al., Mol. Cell. Biol., 4:399-406 (1984)), T7-based expression vector for expression in bacteria (see Rosenberg et al., Gene, 56:125-135 (1987)) and the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, J. Biol. Chem., 263:3521-3527 (1988)). The DNA segment can be present in the vector operably linked to regulatory elements, e.g., a promoter (e.g., polyhedron, T7 or metallothionein (Mt-I) promoters), or a leader sequence to provide for secretory expression of the polypeptide. The recombinant DNA molecule containing the DNA coding for a neuregulin or a fragment or derivative thereof can be introduced into appropriate host cells by known methods. Suitable host cells include e.g. prokaryotes such as E. coli, Bacillus subtilus, etc., and eukaryote such as animal cells and yeast strains, e.g., S. cerevisiae. Mammalian cells may be preferred such as J558, NSO, SP2-O or CHO. In general, conventional culturing conditions can be employed. See Sambrook, *supra*. Stable transformed or transfected cell lines can then be selected.

The expressed neuregulin or fragment or derivative thereof then can be isolated and purified by known methods. Typically the culture medium is centrifuged and the supernatant purified by affinity or immunoaffinity chromatography, e.g. Protein-A or Protein-G affinity chromatography or an immunoaffinity protocol comprising use of monoclonal antibodies that bind neuregulins.

Neuregulin nucleic acids used in the methods of the invention are typically isolated, meaning the nucleic acids comprise a sequence joined to a nucleotide other than that which it is joined to on a natural chromosome and usually constitute at least about 0.5%, preferably at least about 2%, and more preferably at least about 5% by weight of total nucleic acid present in a given fraction. A partially pure nucleic acid constitutes at least about 10%, preferably at least about 30%, and more preferably at least about 60% by weight of total nucleic acid present in a given fraction. A pure nucleic acid constitutes at least about 80%, preferably at least about 90%, and more preferably at least about 95% by weight of total nucleic acid present in a given fraction.

As discussed above, the present invention includes methods for treating and preventing certain neurological-related injuries and disorders, comprising the administration of an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, to a subject including a mammal, particularly a human, in need of such treatment.

In particular, the invention provides methods for treatment and/or prophylaxis of nerve cell death (degeneration) resulting from hypoxia, hypoglycemia, brain or spinal cord ischemia, brain or spinal cord trauma, stroke, heart attack or drowning. Typical candidates for treatment include e.g. heart attack, stroke and/or persons suffering from cardiac arrest neurological deficits, brain or spinal cord injury patients, patients undergoing major surgery such as heart surgery where brain ischemia is a potential complication and patients such as divers suffering from decompression sickness due to gas emboli in the blood stream. Candidates for treatment also will include those patients undergoing a surgical procedure involving extra-corporal circulation such as e.g. a bypass procedure.

The invention also provides methods for treatment which comprise administration of a neuregulin or fragment or derivative thereof, or nucleic acid

encoding same, to a patient that is undergoing surgery or other procedure where brain or spinal cord ischemia is a potential risk. For example, carotid endarterectomy is a surgical procedure employed to correct atherosclerosis of the carotid arteries. Major risks associated with the procedure include intraoperative embolization and the danger of hypertension in the brain following increased cerebral blood flow, which may result in aneurysm or hemorrhage. Thus, an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, could be administered pre-operatively or peri-operatively to reduce such risks associated with carotid endarterectomy, or other post-surgical neurological deficits.

The invention also is effective to promote and enhance recovery from acute nerve cell death and neurological conditions. Thus, for example, a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, could be administered to promote repair, remodeling or reprogramming to a patient that has suffered from stroke or other neuronal injury, suitably for an extended period as discussed above. A therapeutic agent of the invention also could be administered post-operatively to promote recovery from any neurological deficits that may have occurred to a patient that has undergone surgery.

The invention further includes methods for prophylaxis against neurological deficits resulting from e.g. coronary artery bypass graft surgery and aortic valve replacement surgery, or other procedure involving extra-corporal circulation. Those methods will comprise administering to a patient undergoing such surgical procedures an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, typically either pre-operatively or peri-operatively.

The invention also provides methods for prophylaxis and treatment against neurological injury for patients undergoing myocardial infarction, a procedure that can result in ischemic insult to the patient. Such methods will comprise administering to a patient undergoing such surgical procedure an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, typically either pre-operatively or peri-operatively.

Also provided are methods for treating or preventing neuropathic pain such as may be experienced by cancer patients, persons having diabetes, amputees and other persons who may experience neuropathic pain. These methods for treatment comprise

administration of an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, to a patient in need of such treatment.

The invention also provides methods for treatment and prophylaxis against retinal ischemia or degeneration and resulting visual loss. For example, a neuregulin or fragment or derivative thereof, can be administered parenterally or by other procedure as described herein to a subject a suffering from or susceptible to ischemic insult that may adversely affect retinal function, e.g., significantly elevated intraocular pressures, diseases such as retinal artery or vein occlusion, diabetes or other ischemic ocular-related diseases. Post-ischemic administration also may limit retinal damage. The invention also includes methods for treating and prophylaxis against decreased blood flow or nutrient supply to retinal tissue or optic nerve, or treatment or prophylaxis against retinal trauma or optic nerve injury. Subjects for treatment according to such therapeutic methods of the invention may be suffering or susceptible to retinal ischemia that is associated with atherosclerosis, venous capillary insufficiency, obstructive arterial or venous retinopathies, senile macular degeneration, cystoid macular edema or glaucoma, or the retinal ischemia may be associated with a tumor or injury to the mammal. Intravitreal injection also may be a preferred administration route to provide more direct treatment to the ischemic retina.

The invention further provides a method of treating Korsakoff's disease, a chronic alcoholism-induced condition, comprising administering to a subject including a mammal, particularly a human, an effective amount of a neuregulin or fragment or derivative thereof, in an amount effective to treat the disease. Compounds of the invention are anticipated to have utility for the attenuation of cell loss, hemorrhages and/or amino acid changes associated with Korsakoff's disease.

The invention further includes methods for treating a person suffering from or susceptible to epilepsy, emesis, narcotic withdrawal symptoms and age-dependent dementia, comprising administering to a subject including a mammal, particularly a human, an effective amount of a neuregulin or fragment or derivative thereof, in an amount effective to treat the condition.

It will be appreciated that in some instances a neuregulin or a fragment or derivative thereof will be preferably administered to a subject rather than a neuregulin nucleic acid, particularly where a patient is suffering from or susceptible to an acute

neurological injury that demands immediate therapy. For example, administration of a neuregulin polypeptide may be preferred to a patient suffering from stroke, heart attack, traumatic brain injury and the like where it is desired to deliver the active therapeutic as quickly as possible.

In the therapeutic methods of the invention, neuregulin peptides and nucleic acids may be suitably administered to a subject such as a mammal, particularly a human, by any of a number of routes including parenteral (including subcutaneous, intramuscular, intravenous and intradermal), oral, rectal, nasal, vaginal and optical (including buccal and sublingual) administration. A neuregulin protein or nucleic acid or fragment or derivative thereof may be administered to a subject alone or as part of a pharmaceutical composition, comprising the peptide or nucleic acid together with one or more acceptable carriers and optionally other therapeutic ingredients. The carriers should be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Nucleic acids encoding a neuregulin or a neuregulin fragment or derivative can be administered to a patient by generally known gene therapy procedures. See, for example, WO 90/11092 and WO 93/00051. Thus, for instance, the nucleic acids may be introduced into target cells by any method which will result in the uptake and expression of the nucleic acid by the target cells. These methods can include vectors, liposomes, naked DNA, adjuvant-assisted DNA, catheters, etc. Preferably, the administered nucleic acid codes for an appropriate secretory sequence to promote expression upon administration. Suitable vectors for administering a nucleic acid in accordance with the invention include chemical conjugates such as described in WO 93/04701, which has targeting moiety (e.g. a ligand to a cellular surface receptor), and a nucleic acid binding moiety (e.g. polylysine), viral vector (e.g. a DNA or RNA viral vector), fusion proteins such as described in PCT/US 95/02140 (WO 95/22618) which is a fusion protein containing a target moiety (e.g. an antibody specific for a target cell) and a nucleic acid binding moiety (e.g. a protamine), plasmids, phage, etc. The vectors can be chromosomal, non-chromosomal or synthetic.

Preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include moloney murine leukemia viruses. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox

vectors, herpes virus vectors such as a herpes simplex I virus (HSV) vector [A.I. Geller et al., *J. Neurochem,* 64:487 (1995); F. Lim et al., in *DNA Cloning: Mammalian Systems*, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); A.I. Geller et al., *Proc Natl. Acad. Sci.* U.S.A.:90 7603 (1993); A.I. Geller et al., *Proc Natl. Acad. Sci USA*, 87:1149 (1990)], Adenovirus Vectors [LeGal LaSalle et al., *Science*, 259:988 (1993); Davidson, et al., Nat. Genet., 3:219 (1993); Yang et al., *J. Virol.*, 69:2004 (1995)] and Adeno-associated Virus Vectors [Kaplitt, M.G., et al., *Nat. Genet.*, 8:148 (1994)].

Pox viral vectors introduce the gene into the cell cytoplasm. Avipox virus vectors result in only a short-term expression of the nucleic acid. Adenovirus vectors, adeno-associated virus vectors and herpes simplex virus (HSV) vectors are preferred for introducing the nucleic acid into neural cells. The adenovirus vector results in a shorter term expression (about 2 months) than adeno-associated virus (about 4 months), which in turn is shorter than HSV vectors. The particular vector chosen will depend upon the target cell and the specific condition being treated. The introduction can be by standard techniques, e.g. infection, transfection, transduction or transformation. Examples of modes of gene transfer include e.g., naked DNA, $Ca_3(PO_4)_2$ precipitation, DEAE dextran, electroporation, protoplast fusion, lipofecton, cell microinjection, and viral vectors.

A vector can be employed to target essentially any desired target cell. For example, stereotaxic injection can be used to direct the vectors (e.g. adenovirus, HSV) to a desired location. Additionally, the particles can be delivered by intracerebroventricular (icv) infusion using a minipump infusion system, such as a SynchroMed Infusion System. A method based on bulk flow, termed convection, has also proven effective at delivering large molecules to extended areas of the brain and may be useful in delivering the vector to the target cell (Bobo et al., *Proc. Natl. Acad. Sci. USA*, 91:2076-2080 (1994); Morrison et al., *Am. J. Physiol.*, 266:292-305 (1994)). Other methods that can be used include catheters, intravenous, parenteral, intraperitoneal and subcutaneous injection, and oral or other known routes of administration.

Parenteral formulations for administration of a neuregulin or a fragment or derivative thereof may be in the form of liquid solutions or suspensions; for oral

administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences". Formulations for parenteral administration may, for example, contain as excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes, biocompatible, biodegradable lactide polymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the present factors. Other potentially useful parenteral delivery systems for a neuregulin or fragments or derivatives thereof include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration.

The concentration of a neuregulin or a fragment or derivative thereof, or nucleic acid encoding such polypeptides, administered to a particular subject will vary depending upon a number of issues, including the condition being treated, the mode and site of administration, the age, weight sex and general health of the subject, and other such factors that are recognized by those skilled in the art. Optimal administration rates for a given protocol of administration can be readily determined by those skilled in the art.

All documents mentioned herein are incorporated herein by reference in their entirety. The invention is further illustrated by the following non-limiting Examples.

Example 1 -- In vivo neuroprotection assay

Neuregulins and neuregulin fragments and derivatives can be assessed for neuroprotective efficacy pursuant to the following assay.

Mature male Long-Evans rats (Charles River, 250-350g) are allowed food and water *ad libitum*. Animals are anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and placed in a stereotaxic head holder (David Kopf Instruments, Tujunga, CA). The

dorsal surface of the skull is exposed by midline incision, and a small burr hole (2 mm diameter) is drilled over the right lateral ventricle, 1.6 mm lateral and 0.9 mm posterior to bregma. A stainless steel cannula (I.D. 0.020", O.D. 0.028", 2 cm long) is then inserted stereotaxically into the ventricle to a depth of 4.4 mm beneath the surface of the skull. The tubing is suitably bent at a 90° angle 1-1.6 cm from its tip and connected to polyethylene tubing (I.D. 0.76 mm, O.D. 1.22 mm, 10 cm long) that is connected (by glue) to a mini-osmotic pump (Alzet 1007D, 100 µl fill volume, pump rate = 0.5 µl/hr; Alza Corp., Palo Alto, CA) implanted subcutaneously in the back. The cannula can be suitably fixed to the skull by orthodontic resin (L.D. Culk Co., Milford, DE) bonded to two small machine screws (1/8" stainless steel slotted) inserted in the skull. The pump, tubing, and cannula are primed before insertion with infusate solutions; a 3-0 nylon suture is inserted into the cannula during implantation to prevent obstruction by brain tissue. The wound is closed with 3-0 silk suture and cefazolin (10 mg, i.m.) is administered. After surgery animals are suitably kept in individual cages and fed soft food.

Pumps are filled with vehicle alone (containing 127 mM NaCl, 2.6 mM KCl, 1.2 mM $CaCl_2$, 0.9 mM $MgCl_2$, 4.14 mM HEPES, 3 mM glycerin, 0.001% bovine serum albumin [BSA], and 0.01% fast green), or vehicle neuregulin or fragment or derivative thereof (100 µgm/ml). Heparin can be suitably used at relatively low doses, e.g. about 0.8 units/kg/day which is approximately 250-500 times less than a standard anticoagulant dose.

Three days after cannula implantation, animals are reanesthetized with 2% halothane and given atropine (0.15 mg/kg, i.p.). Animals are then intubated and connected to a ventilator (SAR-830; CWE Inc., Ardmore, PA) delivering 1% halothane/70% nitrous oxide in oxygen. The right femoral artery and vein are cannulated for monitoring of mean arterial blood pressure (MABP; Gould RS3200 Blood Pressure Monitor, Gould Inc., Valley View, OH), and blood sampling. Animals are then paralyzed with pancuronium bromide (0.5 mg/kg, i.v.). Arterial blood gasses (Corning 178 Blood Gas Analyzer, Ciba Corning Diagnostic Corp., Medford, MA), blood glucose (Accu-Check Blood Glucose Analyzer, Boehringer Mannheim, Indianapolis, IN), and hematocrit are measured at least twice during surgery and the immediate post-operative period. The stroke volume and rate of the

ventilator are adjusted to maintain PaO_2 between 100-200 mm Hg and $PaCO_2$ between 30-40 mm Hg. Core body temperature may be monitored by rectal thermocouple (e.g. Model 73ATA, Yellow Springs Instrument Co., Yellow Springs, OH) and maintained between 36-37°C with a homeothermic blanket control unit (Harvard Bioscience, South Natick, MA).

Focal ischemic infarcts are made in the right lateral cerebral cortex in the territory of the middle cerebral artery (MCA) by the method of Chen, et al., *Stroke*, 17:738-743 (1986). Both common carotid arteries are exposed by midline anterior cervical incision. The animal is placed in a lateral position and a 1 cm skin incision is then made at the midpoint between the right lateral canthus and the anterior pinna. The temporal muscle is retracted, and a small (3 mm diameter) craniectomy is made at the junction of the zygoma and squamosal bone using a dental drill cooled with saline. Using a dissecting microscope, the dura can be opened with fine forceps, and the right MCA can be ligated with two 10-0 monofilament nylon ties just above the rhinal fissure and transected between the ties. Both common carotid arteries then can be occluded by microaneurysm clips for 45 minutes. After removal of the clips, return of flow is visualized in the arteries. Anesthesia is maintained for 15 minutes, and animals are returned to individual cages and fed soft food after surgery.

Twenty four hours after cerebral infarction, animals are again weighed, and then sacrificed by rapid decapitation. Brains are removed, inspected visually for the anatomy of the middle cerebral artery as well as for signs of hemorrhage or infection, immersed in cold saline for 10 minutes, and sectioned into six standard coronal slices (each 2 mm thick) using a rodent brain matrix slicer (Systems, Warren, MI). Brains are also examined visually for the presence of dye (fast green) in the cerebral ventricles. Slices are placed in the vital dye 2,3,5-triphenyl tetrazolium chloride (TTC, 2%; Chemical Dynamics Co., NH) at 37°C in the dark for 30 minutes, followed by 10% formalin at room temperature overnight. The outline of right and left cerebral hemispheres as well as that of infarcted tissue, clearly visualizable by lack of TTC staining (Chen et al., *Stroke*, 17:738-743 (1986)), is outlined on the posterior surface of each slice using an image analyzer (MTI videocamera and Sony video monitor connected to a Bioquant IV Image Analysis System run on an EVEREX computer). Infarct volume is calculated as the sum of infarcted area per slice multiplied by slice

thickness. Both the surgeon and image analyzer operator are blinded to the treatment given each animal.

Volumes of infarcts among vehicle vs. neuregulin-treated animals can be compared by unpaired, two-tailed t-tests for each experiment, and by two-way analysis of variance (ANOVA; Exp. X Treatment) for combined data. A subsequent slice-by-slice analysis of infarct area among pooled neuregulin- vs. vehicle-treated animals is suitably done by repeated measures two-way ANOVA (Treatment X Slice). Other anatomical and physiological measurements are compared among GDF-1- vs. vehicle-treated animals by unpaired, two-tailed t-tests using the Bonferroni correction for multiple pairwise comparisons.

Example 2 -- In vivo behavioral assays

For behavioral outcome studies, such as to assess recovery, repair and remodeling promoted by administration of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, a number of assays can be employed such as those described in G. Sinson et al., *J Neurochem*, 65(5):2209-2214 (1995); T.K. McIntosh et al., *Neuroscience*, 28:233-244 (1989); and T.K. McIntosh et al., *J Neurotrauma*, 10:373-384 (1993).

Briefly, one suitable behavioral assay as described in G. Sinson et al., *supra*, entails that test animals (male Sprague-Dawley rats) receive preinjury training in a Morris Water Maze, a circular tank 1 m in diameter that is filled with 18°C water. The water surface is made opaque with a covering of Styrofoam pieces. During training of the animals a submerged platform is present in the maze. Each test animal undergoes 20 training trials over a two day period during which they learn to locate the platform using external visual cues. Immediately following the last training trial, animals are anesthetized and subjected to a lateral (parasagittal) fluid-percussion (FP) brain injury. Briefly, a 5-mm craniectomy is performed over the left parietal cortex, midway between lamda and bregma. A hollow Leur-loc fitting is cemented to the craniectomy site. The injury is delivered after attaching the FP device. The injury should be of moderate severity (2.1-2.3 atm). After injury, the Leur-loc is removed, and the skin is sutured. Normothermia is maintained with warming pads until the animals being to ambulate.

At 72 hours, 1 week or 2 weeks after injury, animals are assessed for their ability to remember the learned task of locating the platform in the MWM. For this evaluation the platform is removed from the maze, and the animal's swimming pattern is suitably recorded with a computerized video system for 1 minute. The maze is separated in zones that are weighed according to the proximity to the platform's location. A memory score is generated by multiplying the weighted numbers by the time the animal spends in each zone and then adding the products.

Animals surviving for 1 or 2 weeks also can undergo evaluation of neurologic motor function. Briefly, one suitable assay provides that animals are scored from 0 (severely impaired) to 4 (normal) for each of the following: (1) left and (2) right forelimb during suspension by the tail; (3) left and (4) right hindlimb flexion when the forelimbs remain on a surface and the hindlimbs are lifted up and back by the tail; the ability to resist lateral pulsion to the (5) left and (6) right; and the ability to stand on an inclined plane in the (7) left, (8) right, and (9) vertical positions. Scores are combined for each of the tests (1) through (9). The observer for the tests should be blinded to the animal's previous treatment.

The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of this disclosure, may make modifications and improvements within the spirit and scope of the invention.

What is claimed is:

1. A method of treating a mammal suffering from or susceptible to stroke, brain or spinal cord injury or ischemia, or heart attack, comprising administering to the mammal a therapeutically effective amount of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin.

2. A method of treating a mammal suffering from or susceptible to optic nerve injury or retinal injury or ischemia, comprising administering to the mammal a therapeutically effective amount of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin.

3. A method of treating a mammal suffering from or susceptible to effects of post-surgical neurological deficits, hypoxia or hypoglycemia, comprising administering to the mammal a therapeutically effective amount of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin.

4. A method of treating a mammal suffering from or susceptible to epilepsy, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Alzheimer's disease, Down's Syndrome, Korsakoff's disease, or age-dependent dementia, comprising administering to the mammal a therapeutically effective amount of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin.

5. The method of claim 1 wherein the neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin is administered after the subject has suffered a stroke, brain or spinal cord injury or ischemia, or heart attack.

6. The method of claim 5 wherein the neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin is administered to the subject for at least about two weeks after the subject has suffered a stroke, brain or spinal cord injury or ischemia, or heart attack.

7. A method of any one of claims 1-6 wherein a neuregulin or a fragment or derivative thereof is administered to the mammal.

8. A method of claim 7 wherein the neuregulin or fragment or derivative thereof comprises an amino acid sequence of the following formula:

WYBAZCX

wherein WYBAZCX is composed of amino acid sequences that include one or more sequences shown in FIGS. 1 through 15 (which includes SEQ ID NOS:2, 4, 5, 8, 9, 12, 14, 15, 18, 19, 22, 23, 26, 27, 30, 33, 35, 38, 39, 41, 44, 45 and 48), wherein W comprises the polypeptide segment F, or is absent; wherein Y comprises the polypeptide segment E, or is absent; wherein Z comprises the polypeptide segment G or is absent; and wherein X comprise a polypeptide segment selected from the group consisting of C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/d D'H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL and C/D C/D' D' HKL, and preferably that either

a) at least one of F, Y, B, A, Z, C or X is of bovine origin; or

b) Y comprises the polypeptide segment E; or

c) X comprises the polypeptide segments C/D HKL, C/D D, C/D' HKL, C/D C/D' HKL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HKL, C/D C/D' D'H, C/D C/D' D HL, C/D C/D' D' HKL, C/D'H, C/D C/D' H or C/D C/D' HL.

9. The method of claim 7 wherein the neuregulin or fragment or derivative thereof a) has at least one of F, Y, B, A, Z, C or X is of bovine origin; or b) Y comprises the polypeptide segment E; or c) X comprises the polypeptide segments C/D HKL, C/D D, C/D' HKL, C/D C/D' HKL, C/D C/D' D, C/D D H, C/D D' HL, C/D D' HKL, C/D C/D' D' H, C/D C/D' D HL, C/D C/D' D' HKL, C/D'H, C/D C/D' H or C/D C/D' HL.

10. The method of claim 7 wherein the neuregulin or fragment or derivative thereof comprises FBA polypeptide segments, FEBA polypeptides segments, EBA polypeptide segments, EBA' polypeptide segments or FEBA' polypeptide segments.

11. A method of claim 7 wherein the neuregulin is encoded by a nucleic acid that comprises one of SEQ ID NOS:49, 51 and 53.

12. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a nucleic acid that comprises a sequence that has at least about 70% sequence identity to one of SEQ ID NOS:49, 51 and 53.

13. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a sequence that hybridizes to one of SEQ ID NOS:49, 51 or 53 under normal stringency conditions.

14. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a sequence that hybridizes to one of SEQ ID NOS:49, 51 or 53 under high stringency conditions.

15. A method of claim 7 wherein the neuregulin or fragment or derivative has at least about 70% sequence identity to SEQ ID NOS:50, 52 or 54.

16. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a nucleic acid that comprises a sequence that has at least about 70% sequence identity to one of SEQ ID NO:20 (Figure 7); SEQ ID NO:21 (Figure 7); SEQ ID NO:24 (Figure 8); SEQ ID NO:25 (Figure 8); SEQ ID NO:28 (Figure 9); or SEQ ID NO:29 (Figure 9).

17. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a sequence that hybridizes to one of SEQ ID NO:20 (Figure 7); SEQ ID NO:21 (Figure 7); SEQ ID NO:24 (Figure 8); SEQ ID NO:25 (Figure 8); SEQ ID NO:28 (Figure 9); or SEQ ID NO:29 (Figure 9) under normal stringency conditions.

18. A method of claim 7 wherein the neuregulin or fragment or derivative comprises a sequence that has at least about 70% sequence identity to any of the peptide sequences shown in Figures 7, 8 or 9 of the drawings.

19. A method of claim 7 where the neuregulin or fragment or derivative comprises a sequence that has at least about 80 percent homology to any of the peptide sequences shown in Figures 7, 8 or 9.

20. A method of claim 7 where the neuregulin or fragment or derivative comprises a sequence that has at least about 90 percent homology to any of the peptide sequences shown in Figures 7, 8 or 9.

21. A method of claim 7 wherein the neuregulin or fragment or derivative comprises a sequence that has at least about 95 percent homology to any of the peptide sequences shown in Figures 7, 8 or 9.

22. A method of claim 7 wherein the neuregulin or fragment or derivative comprises a sequence that is shown in Figures 7, 8 or 9.

23. A method of any one of claims 1-6 wherein a nucleic acid encoding a neuregulin or a fragment or derivative thereof is administered to the mammal.

24. A method of claim 23 wherein the nucleic acid is SEQ ID NO:49, 51 or 53, or the complement thereof.

25. A method of claim 23 wherein the nucleic or fragment or derivative thereof encodes a neuregulin or neuregulin fragment or derivative that comprises an amino acid sequence of the following formula:

WYBAZCX

wherein WYBAZCX is composed of amino acid sequences that include one or more sequences shown in FIGS. 1 through 15 (which includes SEQ ID NOS:2, 4, 5, 8, 9, 12, 14, 15, 18, 19, 22, 23, 26, 27, 30, 33, 35, 38, 39, 41, 44, 45 and 48), wherein W comprises the polypeptide segment F, or is absent, wherein Y comprises the polypeptide segment E, or is absent; wherein Z comprises the polypeptide segment G or is absent; and wherein X comprise a polypeptide segment selected from the group consisting of C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL and C/D C/D' D' HKL.

26. The method of claim 25 wherein the neuregulin or neuregulin fragment or derivative a) has at least one of F, Y, B, A, Z, C or X is of bovine origin; or b) Y comprises the polypeptide segment E; or c) X comprises the polypeptide segments C/D HKL, C/D D, C/D' HKL, C/D C/D' HKL, C/D C/D' D, C/D D H, C/D D' HL, C/D D' HKL, C/D C/D' D' H, C/D C/D' D HL, C/D C/D' D' HKL, C/D'H, C/D C/D' H or C/D C/D' HL.

27. The method of claim 25 wherein the neuregulin or neuregulin fragment or derivative comprises FBA polypeptide segments, FEBA polypeptides segments,

EBA polypeptide segments, EBA' polypeptide segments or FEBA' polypeptide segments.

28. A method of claim 23 wherein the nucleic acid comprises a sequence that hybridizes to SEQ ID NO:20 (Figure 7); SEQ ID NO:21 (Figure 7); SEQ ID NO:24 (Figure 8); SEQ ID NO:25 (Figure 8); SEQ ID NO:28 (Figure 9); or SEQ ID NO:29 (Figure 9) under normal stringency conditions.

29. A method of claim 23 wherein the nucleic acid comprises a sequence that hybridizes to SEQ ID NO:SEQ ID NO:20 (Figure 7); SEQ ID NO:21 (Figure 7); SEQ ID NO:24 (Figure 8); SEQ ID NO:25 (Figure 8); SEQ ID NO:28 (Figure 9); or SEQ ID NO:29 (Figure 9) under high stringency conditions.

30. A method of claim 23 wherein the nucleic acid comprises a sequence that has at least about 70 percent homology to any of the nucleic acid sequences shown in Figures 7, 8 or 9.

31. A method of claim 23 wherein the nucleic acid comprises a sequence that has at least about 80 percent homology to any of the nucleic acid sequences shown in Figures 7, 8 or 9.

32. A method of claim 23 wherein the nucleic acid comprises a sequence that has at least about 90 percent homology to any of the nucleic acid sequences shown in Figures 7, 8 or 9.

33. A method of claim 23 wherein the nucleic acid comprises a sequence that has at least about 95 percent homology to any of the nucleic acid sequences shown in Figures 7, 8 or 9.

34. A method of claim 23 wherein the nucleic acid comprises a sequence shown in Figures 7, 8 or 9.

35. A method of any one of claims 1-34 wherein the administered neuregulin fragment or derivative, or the administered nucleic acid encodes a neuregulin fragment or derivative exhibits at least about a 10% reduction in infarct volume in an *in vivo* cerebral ischemia assay.

36. A method of any one of claims 1-35 wherein the mammal is a human.

FIG. 1A
HUMAN SEGMENT E: (SEQ ID NOS:1-2)

```
ATG AGA TGG CGA CGC GCC CCG CGC CGC TCC GGG CGT CCC GGC CCC CGG          48
Met Arg Trp Arg Arg Ala Pro Arg Arg Ser Gly Arg Pro Gly Pro Arg
1               5                  10                  15

GCC CAG CGC CCC GGC TCC GCC GCC CGC TCG TCG CCG CCG CTG CCG CTG          96
Ala Gln Arg Pro Gly Ser Ala Ala Arg Ser Ser Pro Pro Leu Pro Leu
            20                  25                  30

CTG CCA CTA CTG CTG CTG CTG GGG ACC GCG GCC CTG GCG CCG GGG GCG         144
Leu Pro Leu Leu Leu Leu Leu Gly Thr Ala Ala Leu Ala Pro Gly Ala
        35                  40                  45

GCG GCC GGC AAC GAG GCG GCT CCC GCG GGG GCC TCG GTG TGC TAC TCG         192
Ala Ala Gly Asn Glu Ala Ala Pro Ala Gly Ala Ser Val Cys Tyr Ser
    50                  55                  60

TCC CCG CCC AGC GTG GGA TCG GTG CAG GAG CTA GCT CAG CGC GCC GCG         240
Ser Pro Pro Ser Val Gly Ser Val Gln Glu Leu Ala Gln Arg Ala Ala
65                  70                  75                  80

GTG GTG ATC GAG GGA AAG GTG CAC CCG CAG CGG CGG CAG CAG GGG GCA         288
Val Val Ile Glu Gly Lys Val His Pro Gln Arg Arg Gln Gln Gly Ala
                85                  90                  95

CTC GAC AGG AAG GCG GCG GCG GCG GCG GGC GAG GCA GGG GCG TGG GGC         336
Leu Asp Arg Lys Ala Ala Ala Ala Ala Gly Glu Ala Gly Ala Trp Gly
            100                 105                 110

GGC GAT CGC GAG CCG CCA GCC GCG GGC CCA CGG GCG CTG GGG CCG CCC         384
Gly Asp Arg Glu Pro Pro Ala Ala Gly Pro Arg Ala Leu Gly Pro Pro
        115                 120                 125

GCC GAG GAG CCG CTG CTC GCC GCC AAC GGG ACC GTG CCC TCT TGG CCC         432
Ala Glu Glu Pro Leu Leu Ala Ala Asn Gly Thr Val Pro Ser Trp Pro
    130                 135                 140

ACC GCC CCG GTG CCC AGC GCC GGC GAG CCC GGG GAG GAG GCG CCC TAT         480
Thr Ala Pro Val Pro Ser Ala Gly Glu Pro Gly Glu Glu Ala Pro Tyr
145                 150                 155                 160

CTG GTG AAG GTG CAC CAG GTG TGG GCG GTG AAA GCC GGG GGC TTG AAG         528
Leu Val Lys Val His Gln Val Trp Ala Val Lys Ala Gly Gly Leu Lys
                165                 170                 175

AAG GAC TCG CTG CTC ACC GTG CGC CTG GGG ACC TGG GGC CAC CCC GCC         576
Lys Asp Ser Leu Leu Thr Val Arg Leu Gly Thr Trp Gly His Pro Ala
            180                 185                 190
```

FIG. 1B

```
TTC CCC TCC TGC GGG AGG CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC          624
Phe Pro Ser Cys Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe
        195                 200                 205

ATG GAG CCC GAC GCC AAC AGC ACC AGC CGC GCG CCG GCC GCC TTC CGA          672
Met Glu Pro Asp Ala Asn Ser Thr Ser Arg Ala Pro Ala Ala Phe Arg
    210                 215                 220

GCC TCT TTC CCC CCT CTG GAG ACG GGC CGG AAC CTC AAG AAG GAG GTC          720
Ala Ser Phe Pro Pro Leu Glu Thr Gly Arg Asn Leu Lys Lys Glu Val
225                 230                 235                 240

AGC CGG GTG CTG TGC AAG CGG TGC G                                        745
Ser Arg Val Leu Cys Lys Arg Cys
                245
```

FIG.2
SEGMENT E: (SEQ ID NOS:3-4)

```
CC CAT CAA GTG TGG GCG GCG AAA GCC GGG GGC TTG AAG AAG GAC TCG          47
   His Gln Val Trp Ala Ala Lys Ala Gly Gly Leu Lys Lys Asp Ser
     1               5                  10                  15

CTG CTC ACC GTG CGC CTG GGC GCC TGG GGC CAC CCC GCC TTC CCC TCC          95
Leu Leu Thr Val Arg Leu Gly Ala Trp Gly His Pro Ala Phe Pro Ser
                20                  25                  30

TGC GGG CGC CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC ATG GAG CCC         143
Cys Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro
            35                  40                  45

GAG GCC AAC AGC AGC GGC GGG CCC GGC CGC CTT CCG AGC CTC CTT CCC         191
Glu Ala Asn Ser Ser Gly Gly Pro Gly Arg Leu Pro Ser Leu Leu Pro
        50                  55                  60

CCC TCT CGA GAC GGG CCG GAA CCT CAA GAA GGA GGT CAG CCG GGT GCT         239
Pro Ser Arg Asp Gly Pro Glu Pro Gln Glu Gly Gly Gln Pro Gly Ala
    65                  70                  75

GTG CAA CGG TGC G                                                   252
Val Gln Arg Cys
 80
```

FIG. 3
SEGMENT B: (SEQ ID NOS:5-8)

```
Leu Pro Pro Arg Leu Lys Glu His Lys Ser Gln Glu Ser Val Ala Gly
CCT TGC CTC CCC GCT TGA AAG AGA TGA AGA GTC AGG AGT CTG TGG CAG          48
||| ||| ||| ||| | | ||| ||| ||| ||| | | | ||| | | | | | |||
CCT TGC CTC CCC GAT TGA AAG AGA TGA AAA GCC AGG AAT CGG CTG CAG
            Q                                       A

Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
GTT CCA AAC TAG TGC TTC GGT GCG AGA CCA GTT CTG AAT ACT CCT CTC          96
||| ||| ||| ||| | | ||| ||| | | | | ||| ||| ||| ||| ||| ||| |||
GTT CCA AAC TAG TCC TTC GGT GTG AAA CCA GTT CTG AAT ACT CCT CTC

Lys Phe Lys Trp Phe Lys Asn Gly Ser Glu Leu Ser Arg Lys Asn Lys
TCA AGT TCA AGT GGT TCA AGA ATG GGA GTG AAT TAA GCC GAA AGA ACA         144
||| | ||| ||| ||| ||| ||| ||| ||| || ||| | | | ||| | | |||
TCA GAT TCA AGT GGT TCA AGA ATG GGA ATG AAT TGA ATC GAA AAA ACA
R                           N           N

Pro Gly Asn Ile Lys Ile Gln Lys Arg Pro Gly
AAC CAC AAA ACA TCA AGA TAC AGA AAA GGC CGG G                        178
||| ||| ||| | ||| ||| ||| | | ||| || | | |
AAC CAC AAA ATA TCA AGA TAC AAA AAA AGC CAG G
                                    K
```

FIG 4
SEGMENT A: (SEQ ID NOS:9-12)

```
  Lys Ser Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly
G AAG TCA GAA CTT CGC ATT AGC AAA GCG TCA CTG GCT GAT TCT GGA          46
| ||| ||| ||| ||| ||| ||| | | ||| || ||| ||| ||| ||| ||| |||
G AAG TCA GAA CTT CGC ATT AAC AAA GCA TCA CTG GCT GAT TCT GGA
                        N

Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser
GAA TAT ATG TGC AAA GTG ATC AGC AAA CTA GGA AAT GAC AGT GCC TCT          94
|| ||| ||| ||| ||| ||| ||| ||| ||| || ||| ||| ||| ||| ||| |||
GAG TAT ATG TGC AAA GTG ATC AGC AAA TTA GGA AAT GAC AGT GCC TCT

Ala Asn Ile Thr Ile Val Glu Ser Asn Ala
GCC AAC ATC ACC ATT GTG GAG TCA AAC G                                 122
||| || ||| ||| || ||| || ||| ||| |
GCC AAT ATC ACC ATC GTG GAA TCA AAC G
```

FIG.5
SEGMENT A': (SEQ ID NOS:13-14)

```
TCTAAAACTA CAGAGACTGT ATTTTCATGA TCATCATAGT TCTGTGAAAT ATACTTAAAC      60

CGCTTTGGTC CTGATCTTGT AGG AAG TCA GAA CTT CGC ATT AGC AAA GCG         110
                          Lys Ser Glu Leu Arg Ile Ser Lys Ala
                            1               5

TCA CTG GCT GAT TCT GGA GAA TAT ATG TGC AAA GTG ATC AGC AAA CTA       158
Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
 10                  15                  20                  25

GGA AAT GAC AGT GCC TCT GCC AAC ATC ACC ATT GTG GAG TCA AAC GGT       206
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Gly
                30                  35                  40

AAG AGA TGC CTA CTG CGT GCT ATT TCT CAG TCT CTA AGA GGA GTG ATC       254
Lys Arg Cys Leu Leu Arg Ala Ile Ser Gln Ser Leu Arg Gly Val Ile
            45                  50                  55

AAG GTA TGT GGT CAC ACT TGAATCACGC AGGTGTGTGA AATCTCATTG              302
Lys Val Cys Gly His Thr
        60

TGAACAAATA AAAATCATGA AAGGAAAACT CTATGTTTGA AATATCTTAT GGGTCCTCCT     362

GTAAAGCTCT TCACTCCATA AGGTGAAATA GACCTGAAAT ATATATAGAT TATTT          417
```

FIG. 6
SEGMENT G: (SEQ ID NOS:15-18)

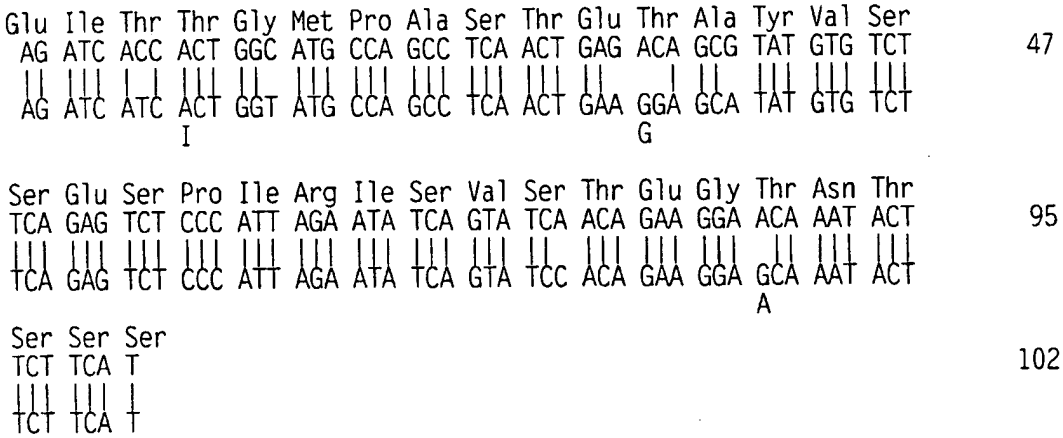

FIG. 7
SEGMENT C: (SEQ ID NOS:19-22)

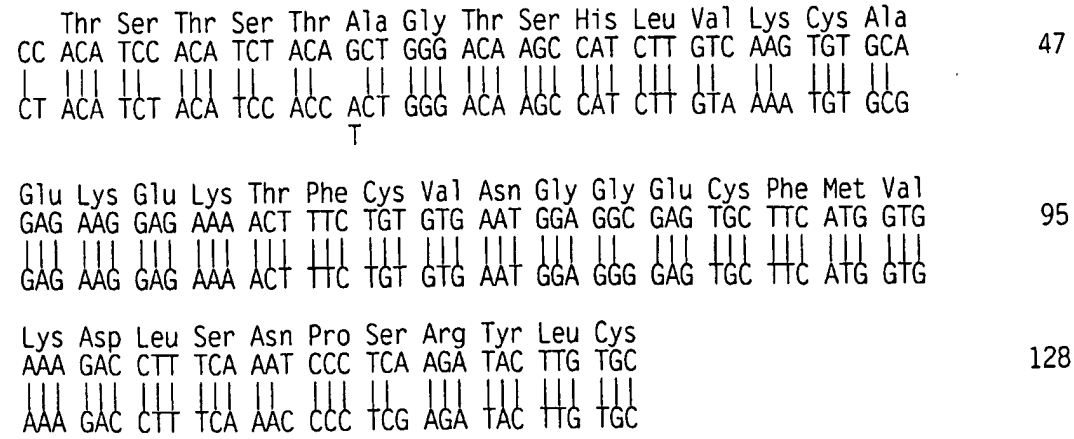

FIG. 8
SEGMENT C/D: (SEQ ID NOS:23-26)

```
Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn Val Pro
AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT GTG CCC      48
||| ||| ||| ||| ||| ||| ||| ||| ||  ||| ||| ||| ||| ||| ||| |||
AAG TGC CAA CCT GGA TTC ACT GGA GCA AGA TGT ACT GAG AAT GTG CCC

Met Lys Val Gln Thr Gln Glu
ATG AAA GTC CAA ACC CAA GAA                                        69
||| ||| ||| ||| |  ||| |||
ATG AAA GTC CAA AAC CAA GAA
              N
```

FIG. 9
SEGMENT C/D': (SEQ ID NOS:27-29)

```
Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val Met
AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG      48
||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG

Ala Ser Phe Tyr
GCC AGC TTC TAC                                                    60
||| ||| ||| |||
GCC AGC TTC TAC
```

FIG. 10
SEGMENT D: (SEQ ID NOS:30-32)

```
Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro Glu  *
AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT GAA TAG                     36
||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT GAA TAG
```

FIG. 11
SEGMENT D': (SEQ ID NOS:33-34)

```
Lys His Leu Gly Ile Glu Phe Met Glu
AAG CAT CTT GGG ATT GAA TTT ATG GAG                    27
```

FIG. 12A
SEGMENT H: (SEQ ID NOS:35-38)

```
Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile
AAA GCG GAG GAG CTC TAC CAG AAG AGA GTG CTC ACC ATT ACC GGC ATT    48
AAA GCG GAG GAG CTG TAC CAG AAG AGA GTG CTG ACC ATA ACC GGC ATC

Cys Ile Ala Leu Leu Val Val Gly Ile Met Cys Val Val Val Tyr Cys
TGC ATC GCG CTG CTC GTG GTT GGC ATC ATG TGT GTG GTG GTC TAC TGC    96
TGC ATC GCC CTC CTT GTG GTC GGC ATC ATG TGT GTG GTG GCC TAC TGC
                                        A

Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp Arg Leu Arg Gln Ser
AAA ACC AAG AAA CAA CGG AAA AAG CTT CAT GAC CGG CTT CGG CAG AGC   144
AAA ACC AAG AAA CAG CGG AAA AAG CTG CAT GAC CGT CTT CGG CAG AGC

Leu Arg Ser Glu Arg Asn Thr Met Met Asn Val Ala Asn Gly Pro His
CTT CGG TCT GAA AGA AAC ACC ATG ATG AAC GTA GCC AAC GGG CCC CAC   192
CTT CGG TCT GAA CGA AAC AAT ATG ATG AAC ATT GCC AAT GGG CCT CAC
                          N             I

His Pro Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr Val
CAC CCC AAT CCG CCC CCC GAG AAC GTG CAG CTG GTG AAT CAA TAC GTA   240
CAT CCT AAC CCA CCC CCC GAG AAT GTC CAG CTG GTG AAT CAA TAC GTA

Ser Lys Asn Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu
TCT AAA AAT GTC ATC TCT AGC GAG CAT ATT GTT GAG AGA GAG GCG GAG   288
TCT AAA AAC GTC ATC TCC AGT GAG CAT ATT GTT GAG AGA GAA GCA GAG

Ser Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr
AGC TCT TTT TCC ACC AGT CAC TAC ACT TCG ACA GCT CAT CAT TCC ACT   336
ACA TCC TTT TCC ACC AGT CAC TAT ACT TCC ACA GCC CAT CAC TCC ACT
T
```

FIG. 12B

```
Thr Val Thr Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr Glu
ACT GTC ACT CAG ACT CCC AGT CAC AGC TGG AGC AAT GGA CAC ACT GAA      384
ACT GTC ACC CAG ACT CCT AGC CAC AGC TGG AGC AAC GGA CAC ACT GAA

Ser Ile Ile Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu
AGC ATC ATT TCG GAA AGC CAC TCT GTC ATC GTG ATG TCA TCC GTA GAA      432
AGC ATC CTT TCC GAA AGC CAC TCT GTA ATC GTG ATG TCA TCC GTA GAA
      L

Asn Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn
AAC AGT AGG CAC AGC AGC CCG ACT GGG GGC CCG AGA GGA CGT CTC AAT      480
AAC AGT AGG CAC AGC AGC CCA ACT GGG GGC CCA AGA GGA CGT CTT AAT

Gly Leu Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala Arg
GGC TTG GGA GGC CCT CGT GAA TGT AAC AGC TTC CTC AGG CAT GCC AGA      528
GGC ACA GGA GGC CCT CGT GAA TGT AAC AGC TTC CTC AGG CAT GCC AGA
    T

Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser Glu Arg
GAA ACC CCT GAC TCC TAC CGA GAC TCT CCT CAT AGT GAA AG              569
GAA ACC CCT GAT TCC TAC CGA GAC TCT CCT CAT AGT GAA AG
```

FIG. 13
SEGMENT K: (SEQ ID NOS:39-40)

```
A CAT AAC CTT ATA GCT GAG CTA AGG AGA AAC AAG GCC CAC AGA TCC       46
  His Asn Leu Ile Ala Glu Leu Arg Arg Asn Lys Ala His Arg Ser
    1               5                  10                  15

AAA TGC ATG CAG ATC CAG CTT TCC GCA ACT CAT CTT AGA GCT TCT TCC     94
Lys Cys Met Gln Ile Gln Leu Ser Ala Thr His Leu Arg Ala Ser Ser
                20                  25                  30

ATT CCC CAT TGG GCT TCA TTC TCT AAG ACC CCT TGG CCT TTA GGA AG     141
Ile Pro His Trp Ala Ser Phe Ser Lys Thr Pro Trp Pro Leu Gly Arg
            35                  40                  45
```

FIG. 14A
SEGMENT L: (SEQ ID NOS:41-44)

```
  Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro Val Asp
G TAT GTA TCA GCA ATG ACC ACC CCG GCT CGT ATG TCA CCT GTA GAT          46
| ||| || ||| || ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
G TAT GTG TCA GCC ATG ACC ACC CCG GCT CGT ATG TCA CCT GTA GAT

Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro Ser Glu Met Ser Pro
TTC CAC ACG CCA AGC TCC CCC AAG TCA CCC CCT TCG GAA ATG TCC CCG          94
||| ||| ||| ||| ||| ||| ||| || || ||| ||| ||| ||| ||| ||| ||
TTC CAC ACG CCA AGC TCC CCC AAA TCG CCC CCT TCG GAA ATG TCT CCA

Pro Val Ser Ser Thr Thr Val Ser Met Pro Ser Met Ala Val Ser Pro
CCC GTG TCC AGC ACG ACG GTC TCC ATG CCC TCC ATG GCG GTC AGT CCC         142
||| ||| ||| ||| | | ||| || ||| ||| || ||| ||| ||| ||| || |||
CCC GTG TCC AGC ATG ACG GTG TCC ATG CCT TCC ATG GCG GTC AGC CCC
                M

Phe Val Glu Glu Glu Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu
TTC GTG GAA GAG GAG AGA CCC CTG CTC CTT GTG ACG CCA CCA CGG CTG         190
||| || ||| || ||| ||| || || || || ||| || ||| ||| || |||
TTC ATG GAA GAA GAG AGA CCT CTA CTT CTC GTG ACA CCA CCA AGG CTG
    N

Arg Glu Lys  -  Tyr Asp His His Ala Gln Gln Phe Asn Ser Phe His
CGG GAG AAG ... TAT GAC CAC CAC GCC CAG CAA TTC AAC TCG TTC CAC         238
||| ||| |||     || ||| || ||| |  ||| || ||| | || ||| |||
CGG GAG AAG AAG TTT GAC CAT CAC CCT CAG CAG TTC AGC TCC TTC CAC
            K   F             P

Cys Asn Pro Ala His Glu Ser Asn Ser Leu Pro Pro Ser Pro Leu Arg
TGC AAC CCC GCG CAT GAG AGC AAC AGC CTG CCC CCC AGC CCC TTG AGG         286
 |  ||| ||| ||| ||| || || ||| ||| || || |  ||| ||| ||| |||
CAC AAC CCC GCG CAT GAC AGT AAC AGC CTC CCT GCT AGC CCC TTG AGG
N               D                       A

Ile Val Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala
ATA GTG GAG GAT GAG GAA TAT GAA ACG ACC CAG GAG TAC GAA CCA GCT         334
||| ||| ||| ||| ||| || ||| ||| ||| ||| || ||| ||| || ||| ||
ATA GTG GAG GAT GAG GAG TAT GAA ACG ACC CAA GAG TAC GAG CCA GCC

Gln Glu Pro Val Lys Lys Leu Thr Asn Ser Ser Arg Arg Ala Lys Arg
CAA GAG CCG GTT AAG AAA CTC ACC AAC AGC AGC CGG CGG GCC AAA AGA         382
||| ||| ||  ||| ||| ||| ||| || ||| .  ||| ||| ||| ||| ||| |||
CAA GAG CCT GTT AAG AAA CTC GCC AA. ..T AGC CGG CGG GCC AAA AGA
                            A
```

FIG. 14B

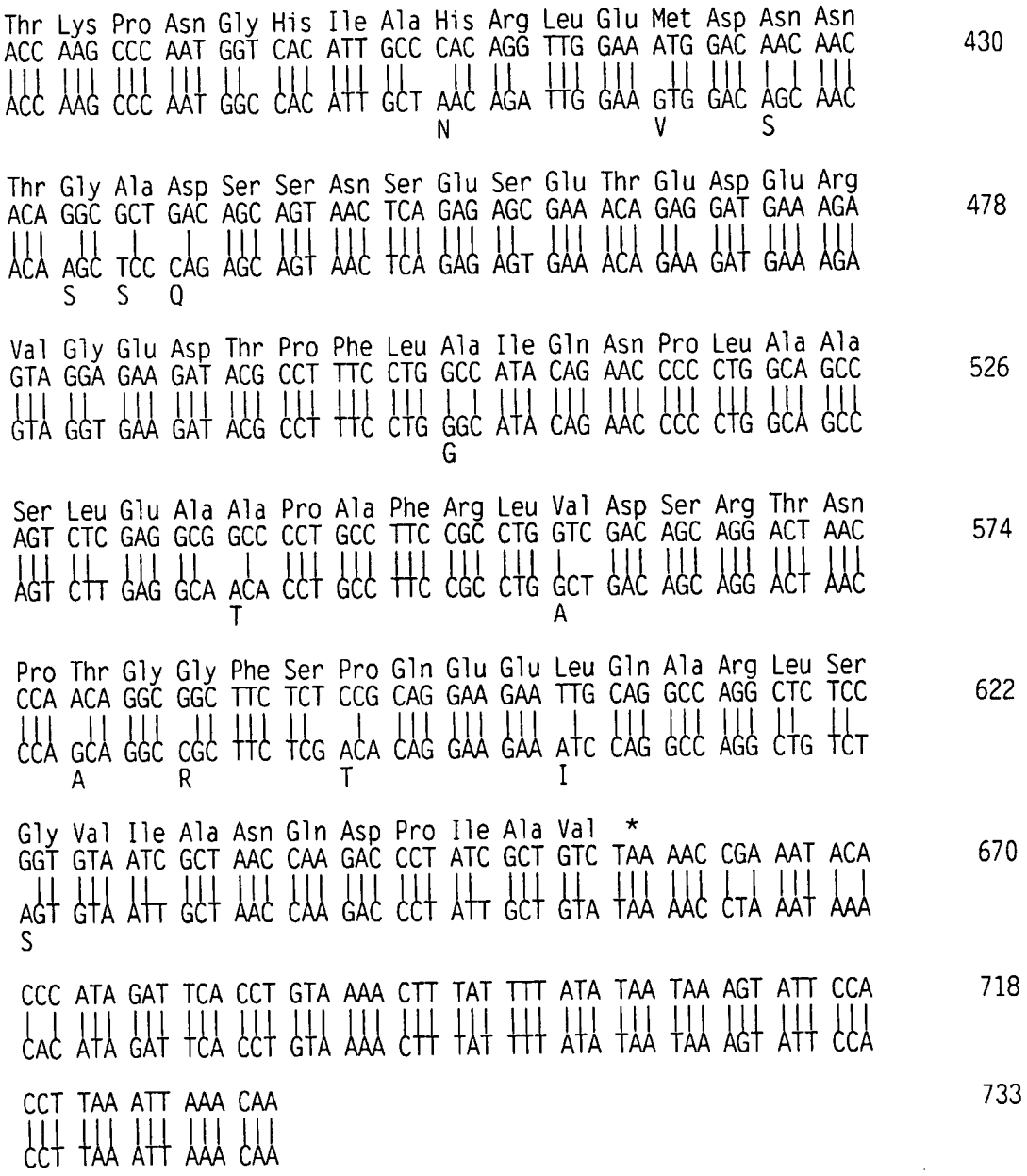
Thr Lys Pro Asn Gly His Ile Ala His Arg Leu Glu Met Asp Asn Asn
ACC AAG CCC AAT GGT CAC ATT GCC CAC AGG TTG GAA ATG GAC AAC AAC
ACC AAG CCC AAT GGC CAC ATT GCT AAC AGA TTG GAA GTG GAC AGC AAC
N V S
430
Thr Gly Ala Asp Ser Ser Asn Ser Glu Ser Glu Thr Glu Asp Glu Arg
ACA GGC GCT GAC AGC AGT AAC TCA GAG AGC GAA ACA GAG GAT GAA AGA
ACA AGC TCC CAG AGC AGT AAC TCA GAG AGT GAA ACA GAA GAT GAA AGA
S S Q
478
Val Gly Glu Asp Thr Pro Phe Leu Ala Ile Gln Asn Pro Leu Ala Ala
GTA GGA GAA GAT ACG CCT TTC CTG GCC ATA CAG AAC CCC CTG GCA GCC
GTA GGT GAA GAT ACG CCT TTC CTG GGC ATA CAG AAC CCC CTG GCA GCC
G
526
Ser Leu Glu Ala Ala Pro Ala Phe Arg Leu Val Asp Ser Arg Thr Asn
AGT CTC GAG GCG GCC CCT GCC TTC CGC CTG GTC GAC AGC AGG ACT AAC
AGT CTT GAG GCA ACA CCT GCC TTC CGC CTG GCT GAC AGC AGG ACT AAC
T A
574
Pro Thr Gly Gly Phe Ser Pro Gln Glu Glu Leu Gln Ala Arg Leu Ser
CCA ACA GGC GGC TTC TCT CCG CAG GAA GAA TTG CAG GCC AGG CTC TCC
CCA GCA GGC CGC TTC TCG ACA CAG GAA GAA ATC CAG GCC AGG CTG TCT
A R T I
622
Gly Val Ile Ala Asn Gln Asp Pro Ile Ala Val *
GGT GTA ATC GCT AAC CAA GAC CCT ATC GCT GTC TAA AAC CGA AAT ACA
AGT GTA ATT GCT AAC CAA GAC CCT ATT GCT GTA TAA AAC CTA AAT AAA
S
670
CCC ATA GAT TCA CCT GTA AAA CTT TAT TTT ATA TAA TAA AGT ATT CCA
CAC ATA GAT TCA CCT GTA AAA CTT TAT TTT ATA TAA TAA AGT ATT CCA
718
CCT TAA ATT AAA CAA
CCT TAA ATT AAA CAA
733

FIG. 15
SEGMENT F: (SEQ ID NOS:45-48)

```
AGTTTCCCCC CCCAACTTGT CGGAACTCTG GGCTCGCGCG CAGGGCAGGA GCGGAGCGGC      60

GGCGGCTGCC CAGGCGATGC GAGCGCGGGC CGGACGGTAA TCGCCTCTCC CTCCTCGGGC     120

TGCGAGCGCG CCGGACCGAG GCAGCGACAG GAGCGGACCG CGGCGGGAAC CGAGGACTCC     180

CCAGCGGCGC GCCAGCAGGA GCCACCCCGC GAGNCGTGCG ACCGGGACGG AGCGCCCGCC     240

AGTCCCAGGT GGCCCGGACC GCACGTTGCG TCCCCGCGCT CCCCGCCGGC GACAGGAGAC     300

GCTCCCCCCC ACGCCGCGCG CGCCTCGGCC CGGTCGCTGG CCCGCCTCCA CTCCGGGGAC     360
                |||| | |||||| || ||| |||| | |||| || ||| |||||
               CGCGAG CGCCTCAGCG CGGCCGCTCG CTCTC..CCC CTCGAGGGAC

AAACTTTTCC CGAAGCCGAT CCCAGCCCTC GGACCCAAAC TTGTCGCGCG TCGCCTTCGC     420
|||||||||| | || |||||| || ||||||| |||||| ||          |||||| |||
AAACTTTTCC CAAACCCGAT CCGAGCCCTT GGACCAAA.. .........C TCGCCTGCGC

                                                Met Ser Glu Arg Arg
CGGGAGCCGT CCGCGCAGAG CGTGCACTTC TCGGGCGAG ATG TCG GAG CGC AGA       474
|| |||||||| ||||| |||| || | ||| || ||||||| ||| || ||| ||| | |
CGAGAGCCGT CCGCGTAGAG CGCTC.CGTC TCCGGCGAG ATG TCC GAG CGC AAA
                                                          K

Glu Gly Lys Gly Lys Gly Lys Gly Gly Lys Lys Asp Arg Gly Ser Gly
GAA GGC AAA GGC AAG GGG AAG GGC GGC AAG AAG GAC CGA GGC TCC GGG      522
||| ||| | | ||| || ||| ||| |||     ||| ||| || ||| ||| ||| ||
GAA GGC AGA GGC AAA GGG AAG GGC AAG AAG AAG GAG CGA GGC TCC GGC
        R                     K           E

Lys Lys Pro Val Pro Ala Ala Gly Gly Pro Ser Pro Ala
AAG AAG CCC GTG CCC GCG GCT GGC GGC CCG AGC CCA G                   559
||| ||| || | | || ||| || ||| || | | ||| ||| |
AAG AAG CCG GAG TCC GCG GCG GGC AGC CAG AGC CCA G
          E   S
```

FIG. 16A
(SEQ ID NOS:49-50)

```
G AAG TCA GAA CTT CGC ATT AGC AAA GCG TCA CTG GCT GAT TCT GGA GAA      49
  Lys Ser Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu
   1               5                  10                  15

TAT ATG TGC AAA GTG ATC AGC AAA CTA GGA AAT GAC AGT GCC TCT GCC      97
Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala
            20                  25                  30

AAC ATC ACC ATT GTG GAG TCA AAC GCC ACA TCC ACA TCT ACA GCT GGG     145
Asn Ile Thr Ile Val Glu Ser Asn Ala Thr Ser Thr Ser Thr Ala Gly
        35                  40                  45

ACA AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG     193
Thr Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val
    50                  55                  60

AAT GGA GGC GAC TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA     241
Asn Gly Gly Asp Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg
65                  70                  75                  80

TAC TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG     289
Tyr Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu
                85                  90                  95

AAT GTG CCC ATG AAA GTC CAA ACC CAA GAA AAA GCG GAG GAG CTC TAC     337
Asn Val Pro Met Lys Val Gln Thr Gln Glu Lys Ala Glu Glu Leu Tyr
            100                 105                 110

CAG AAG AGA GTG CTC ACC ATT ACC GGC ATT TGC ATC GCG CTG CTC GTG     385
Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val
        115                 120                 125

GTT GGC ATC ATG TGT GTG.GTG GTC TAC TGC AAA ACC AAG AAA CAA CGG     433
Val Gly Ile Met Cys Val Val Val Tyr Cys Lys Thr Lys Lys Gln Arg
    130                 135                 140

AAA AAG CTT CAT GAC CGG CTT CGG CAG AGC CTT CGG TCT GAA AGA AAC     481
Lys Lys Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn
145                 150                 155                 160

ACC ATG ATG AAC GTA GCC AAC GGG CCC CAC CAC CCC AAT CCG CCC CCC     529
Thr Met Met Asn Val Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
                165                 170                 175

GAG AAC GTG CAG CTG GTG AAT CAA TAC GTA TCT AAA AAT GTC ATC TCT     577
Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile Ser
            180                 185                 190
```

FIG. 16B

```
AGC GAG CAT ATT GTT GAG AGA GAG GCG GAG AGC TCT TTT TCC ACC AGT          625
Ser Glu His Ile Val Glu Arg Glu Ala Glu Ser Ser Phe Ser Thr Ser
        195                 200                 205

CAC TAC ACT TCG ACA GCT CAT CAT TCC ACT ACT GTC ACT CAG ACT CCC          673
His Tyr Thr Ser Thr Ala His His Ser Thr Thr Val Thr Gln Thr Pro
    210                 215                 220


AGT CAC AGC TGG AGC AAT GGA CAC ACT GAA AGC ATC ATT TCG GAA AGC          721
Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile Ile Ser Glu Ser
225                 230                 235                 240

CAC TCT GTC ATC GTG ATG TCA TCC GTA GAA AAC AGT AGG CAC AGC AGC          769
His Ser Val Ile Val Met Ser Ser Val Glu Asn Ser Arg His Ser Ser
                245                 250                 255

CCG ACT GGG GGC CCG AGA GGA CGT CTC AAT GGC TTG GGA GGC CCT CGT          817
Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly Leu Gly Gly Pro Arg
            260                 265                 270

GAA TGT AAC AGC TTC CTC AGG CAT GCC AGA GAA ACC CCT GAC TCC TAC          865
Glu Cys Asn Ser Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr
        275                 280                 285

CGA GAC TCT CCT CAT AGT GAA AGA CAT AAC CTT ATA GCT GAG CTA AGG          913
Arg Asp Ser Pro His Ser Glu Arg His Asn Leu Ile Ala Glu Leu Arg
    290                 295                 300

AGA AAC AAG GCC CAC AGA TCC AAA TGC ATG CAG ATC CAG CTT TCC GCA          961
Arg Asn Lys Ala His Arg Ser Lys Cys Met Gln Ile Gln Leu Ser Ala
305                 310                 315                 320

ACT CAT CTT AGA GCT TCT TCC ATT CCC CAT TGG GCT TCA TTC TCT AAG         1009
Thr His Leu Arg Ala Ser Ser Ile Pro His Trp Ala Ser Phe Ser Lys
                325                 330                 335

ACC CCT TGG CCT TTA GGA AGG TAT GTA TCA GCA ATG ACC ACC CCG GCT         1057
Thr Pro Trp Pro Leu Gly Arg Tyr Val Ser Ala Met Thr Thr Pro Ala
            340                 345                 350

CGT ATG TCA CCT GTA GAT TTC CAC ACG CCA AGC TCC CCC AAG TCA CCC         1105
Arg Met Ser Pro Val Asp Phe His Thr Pro Ser Ser Pro Lys Ser Pro
        355                 360                 365

CCT TCG GAA ATG TCC CCG CCC GTG TCC AGC ACG ACG GTC TCC ATG CCC         1153
Pro Ser Glu Met Ser Pro Pro Val Ser Ser Thr Thr Val Ser Met Pro
    370                 375                 380
```

FIG. 16C

```
TCC ATG GCG GTC AGT CCC TTC GTG GAA GAG GAG AGA CCC CTG CTC CTT      1201
Ser Met Ala Val Ser Pro Phe Val Glu Glu Glu Arg Pro Leu Leu Leu
385                 390                 395                 400

GTG ACG CCA CCA CGG CTG CGG GAG AAG TAT GAC CAC CAC GCC CAG CAA      1249
Val Thr Pro Pro Arg Leu Arg Glu Lys Tyr Asp His His Ala Gln Gln
                405                 410                 415

TTC AAC TCG TTC CAC TGC AAC CCC GCG CAT GAG AGC AAC AGC CTG CCC      1297
Phe Asn Ser Phe His Cys Asn Pro Ala His Glu Ser Asn Ser Leu Pro
            420                 425                 430

CCC AGC CCC TTG AGG ATA GTG GAG GAT GAG GAA TAT GAA ACG ACC CAG      1345
Pro Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr Glu Thr Thr Gln
        435                 440                 445

GAG TAC GAA CCA GCT CAA GAG CCG GTT AAG AAA CTC ACC AAC AGC AGC      1393
Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys Leu Thr Asn Ser Ser
    450                 455                 460

CGG CGG GCC AAA AGA ACC AAG CCC AAT GGT CAC ATT GCC CAC AGG TTG      1441
Arg Arg Ala Lys Arg Thr Lys Pro Asn Gly His Ile Ala His Arg Leu
465                 470                 475                 480

GAA ATG GAC AAC AAC ACA GGC GCT GAC AGC AGT AAC TCA GAG AGC GAA      1489
Glu Met Asp Asn Asn Thr Gly Ala Asp Ser Ser Asn Ser Glu Ser Glu
                485                 490                 495

ACA GAG GAT GAA AGA GTA GGA GAA GAT ACG CCT TTC CTG GCC ATA CAG      1537
Thr Glu Asp Glu Arg Val Gly Glu Asp Thr Pro Phe Leu Ala Ile Gln
            500                 505                 510

AAC CCC CTG GCA GCC AGT CTC GAG GCG GCC CCT GCC TTC CGC CTG GTC      1585
Asn Pro Leu Ala Ala Ser Leu Glu Ala Ala Pro Ala Phe Arg Leu Val
        515                 520                 525

GAC AGC AGG ACT AAC CCA ACA GGC GGC TTC TCT CCG CAG GAA GAA TTG      1633
Asp Ser Arg Thr Asn Pro Thr Gly Gly Phe Ser Pro Gln Glu Glu Leu
    530                 535                 540

CAG GCC AGG CTC TCC GGT GTA ATC GCT AAC CAA GAC CCT ATC GCT GTC      1681
Gln Ala Arg Leu Ser Gly Val Ile Ala Asn Gln Asp Pro Ile Ala Val
545                 550                 555                 560

TAAAACCGAA ATACACCCAT AGATTCACCT GTAAAACTTT ATTTTATATA ATAAAGTATT    1741

CCACCTTAAA TTAAACAAAA AAA                                           1764
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FIG. 17A
(SEQ ID NOS:51-52)

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CAT CAA GTG TGG GCG GCG AAA GCC GGG GGC TTG AAG AAG GAC TCG CTG          48
His Gln Val Trp Ala Ala Lys Ala Gly Gly Leu Lys Lys Asp Ser Leu
1               5                  10                  15

CTC ACC GTG CGC CTG GGC GCC TGG GGC CAC CCC GCC TTC CCC TCC TGC          96
Leu Thr Val Arg Leu Gly Ala Trp Gly His Pro Ala Phe Pro Ser Cys
            20                  25                  30

GGG CGC CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC ATG GAG CCC GAG         144
Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro Glu
        35                  40                  45

GCC AAC AGC AGC GGC GGG CCC GGC CGC CTT CCG AGC CTC CTT CCC CCC         192
Ala Asn Ser Ser Gly Gly Pro Gly Arg Leu Pro Ser Leu Leu Pro Pro
    50                  55                  60

TCT CGA GAC GGG CCG GAA CCT CAA GAA GGA GGT CAG CCG GGT GCT GTG         240
Ser Arg Asp Gly Pro Glu Pro Gln Glu Gly Gly Gln Pro Gly Ala Val
65                  70                  75                  80

CAA CGG TGC GCC TTG CCT CCC CGC TTG AAA GAG ATG AAG AGT CAG GAG         288
Gln Arg Cys Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
                85                  90                  95

TCT GTG GCA GGT TCC AAA CTA GTG CTT CGG TGC GAG ACC AGT TCT GAA         336
Ser Val Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu
            100                 105                 110

TAC TCC TCT CTC AAG TTC AAG TGG TTC AAG AAT GGG AGT GAA TTA AGC         384
Tyr Ser Ser Leu Lys Phe Lys Trp Phe Lys Asn Gly Ser Glu Leu Ser
        115                 120                 125

CGA AAG AAC AAA CCA GAA AAC ATC AAG ATA CAG AAA AGG CCG GGG AAG         432
Arg Lys Asn Lys Pro Glu Asn Ile Lys Ile Gln Lys Arg Pro Gly Lys
    130                 135                 140

TCA GAA CTT CGC ATT AGC AAA GCG TCA CTG GCT GAT TCT GGA GAA TAT         480
Ser Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr
145                 150                 155                 160

ATG TGC AAA GTG ATC AGC AAA CTA GGA AAT GAC AGT GCC TCT GCC AAC         528
Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn
                165                 170                 175
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FIG. 17B

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ATC ACC ATT GTG GAG TCA AAC GCC ACA TCC ACA TCT ACA GCT GGG ACA          576
Ile Thr Ile Val Glu Ser Asn Ala Thr Ser Thr Ser Thr Ala Gly Thr
            180                 185                 190

AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT          624
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
        195                 200                 205

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC          672
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
    210                 215                 220

TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT          720
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn
225                 230                 235                 240

GTG CCC ATG AAA GTC CAA ACC CAA GAA AAG TGC CCA AAT GAG TTT ACT          768
Val Pro Met Lys Val Gln Thr Gln Glu Lys Cys Pro Asn Glu Phe Thr
                245                 250                 255

GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AGT ACG TCC          816
Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser
            260                 265                 270

ACT CCC TTT CTG TCT CTG CCT GAA TAGCGCATCT CAGTCGGTGC CGCTTTCTTG         870
Thr Pro Phe Leu Ser Leu Pro Glu
        275                 280

TTGCCGCATC TCCCCTCAGA TTCCNCCTAG AGCTAGATGC GTTTTACCAG GTCTAACATT        930

GACTGCCTCT GCCTGTCGCA TGAGAACATT AACACAAGCG ATTGTATGAC TTCCTCTGTC        990

CGTGACTAGT GGGCTCTGAG CTACTCGTAG GTGCGTAAGG CTCCAGTGTT TCTGAAATTG       1050

ATCTTGAATT ACTGTGATAC GACATGATAG TCCCTCTCAC CCAGTGCAAT GACAATAAAG       1110

GCCTTGAAAA GTCAAAAAAA AAAAAAAAAA                                        1140
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FIG. 18A
(SEQ ID NOS:53-54)

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AGTTTCCCCC CCCAACTTGT CGGAACTCTG GGCTCGCGCG CAGGGCAGGA GCGGAGCGGC      60

GGCGGCTGCC CAGGCGATGC GAGCGCGGGC CGGACGGTAA TCGCCTCTCC CTCCTCGGGC     120

TGCGAGCGCG CCGGACCGAG GCAGCGACAG GAGCGGACCG CGGCGGGAAC CGAGGACTCC     180

CCAGCGGCGC GCCAGCAGGA GCCACCCCGC GAGCGTGCGA CCGGGACGGA GCGCCCGCCA     240

GTCCCAGGTG GCCCGGACCG CACGTTGCGT CCCCGCGCTC CCCGCCGGCG ACAGGAGACG     300

CTCCCCCCCA CGCCGCGCGC GCCTCGGCCC GGTCGCTGGC CCGCCTCCAC TCCGGGGACA     360

AACTTTTCCC GAAGCCGATC CCAGCCCTCG GACCCAAACT TGTCGCGCGT CGCCTTCGCC     420

GGGAGCCGTC CGCGCAGAGC GTGCACTTCT CGGGCGAG ATG TCG GAG CGC AGA          473
                                          Met Ser Glu Arg Arg
                                            1               5

GAA GGC AAA GGC AAG GGG AAG GGC GGC AAG AAG GAC CGA GGC TCC GGG        521
Glu Gly Lys Gly Lys Gly Lys Gly Gly Lys Lys Asp Arg Gly Ser Gly
                  10                  15                  20

AAG AAG CCC GTG CCC GCG GCT GGC GGC CCG AGC CCA GCC TTG CCT CCC        569
Lys Lys Pro Val Pro Ala Ala Gly Gly Pro Ser Pro Ala Leu Pro Pro
          25                  30                  35

CGC TTG AAA GAG ATG AAG ATG CAG GAG TCT GTG GCA GGT TCC AAA CTA        617
Arg Leu Lys Glu Met Lys Ser Gln Glu Ser Val Ala Gly Ser Lys Leu
        40                  45                  50

GTG CTT CGG TGC GAG ACC AGT TCT GAA TAC TCC TCT CTC AAG TTC AAG        665
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu Lys Phe Lys
     55                  60                  65

TGG TTC AAG AAT GGG AGT GAA TTA AGC CGA AAG AAC AAA CCA CAA AAC        713
Trp Phe Lys Asn Gly Ser Glu Leu Ser Arg Lys Asn Lys Pro Gln Asn
 70                  75                  80                  85

ATC AAG ATA CAG AAA AGG CCG GGG AAG TCA GAA CTT CGC ATT AGC AAA        761
Ile Lys Ile Gln Lys Arg Pro Gly Lys Ser Glu Leu Arg Ile Ser Lys
                  90                  95                 100

GCG TCA CTG GCT GAT TCT GGA GAA TAT ATG TGC AAA GTG ATC AGC AAA        809
Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys
          105                 110                 115
```

FIG. 18B

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CTA GGA AAT GAC AGT GCC TCT GCC AAC ATC ACC ATT GTG GAG TCA AAC        857
Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn
        120                 125                 130

GAG ATC ACC ACT GGC ATG CCA GCC TCA ACT GAG ACA GCG TAT GTG TCT        905
Glu Ile Thr Thr Gly Met Pro Ala Ser Thr Glu Thr Ala Tyr Val Ser
    135                 140                 145

TCA GAG TCT CCC ATT AGA ATA TCA GTA TCA ACA GAA GGA ACA AAT ACT        953
Ser Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Thr Asn Thr
150                 155                 160                 165

TCT TCA TCC ACA TCC ACA TCT ACA GCT GGG ACA AGC CAT CTT GTC AAG       1001
Ser Ser Ser Thr Ser Thr Ser Thr Ala Gly Thr Ser His Leu Val Lys
                170                 175                 180

TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGC GAG TGC TTC       1049
Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys Phe
            185                 190                 195

ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC TTG TGC AAG TGC CCA       1097
Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro
        200                 205                 210

AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC       1145
Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe
    215                 220                 225

TAC AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT GAA TAGGCGCATG            1191
Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro Glu
230                 235                 240

CTCAGTCGGT GCCGCTTTCT TGTTGCCGCA TCTCCCCTCA GATTCAACCT AGAGCTAGAT    1251

GCGTTTTACC AGGTCTAACA TTGACTGCCT CTGCCTGTCG CATGAGAACA TTAACACAAG    1311

CGATTGTATG ACTTCCTCTG TCCGTGACTA GTGGGCTCTG AGCTACTCGT AGGTGCGTAA    1371

GGCTCCAGTG TTTCTGAAAT TGATCTTGAA TTACTGTGAT ACGACATGAT AGTCCCTCTC    1431

ACCCAGTGCA ATGACAATAA AGGCCTTGAA AAGTCTCACT TTTATTGAGA AAATAAAAAT    1491

CGTTCCACGG GACAGTCCCT CTTCTTTATA AAATGACCCT ATCCTTGAAA AGGAGGTGTG    1551

TTAAGTTGTA ACCAGTACAC ACTTGAAATG ATGGTAAGTT CGCTTCGGTT CAGAATGTGT    1611

TCTTTCTGAC AAATAAACAG AATAAAAAAA AAAAAAAAAA A                        1652
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21349

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/70, 38/00, 38/02, 38/18
US CL :514/2, 12, 44, 903, 907

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 12, 44, 903, 907

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,530,109 A (GOODEARL et al.) 25 June 1996, column 3, line 3 to column 6, line 53 and column 11, line 1 to column 12, line 59.	1-34

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
17 DECEMBER 1998	05FEB1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer STEPHEN GUCKER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21349

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 35-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21349

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, SCISEARCH, EMBASE, BIOSIS, CAPLUS, WPIDS, BIOTECHDS, CONFSCI, LIFESCI
neuregulin#, glia#, heregulin#, GGF#, ischemia#, dementia#, Parkinson#, Huntington#, Alzheimer#, infarct#, amyotrophic, Down#, Korsakoff#, heart#, cardiac, spinal